Chapter 3
Design of PE Samples

This chapter outlines general and specific design considerations for PE samples including customizing samples.

3.1. General Design Considerations.

General design considerations for PE samples include the following:

- Determine the use of PE samples early in project planning to allow adequate time for selection or design of samples.
- Define clear goals for PE samples around the project's analysis needs and DQO.
- Design PE samples so that the entire laboratory is evaluated according to PE sample goals.
- Provide consistent but also project-specific challenges to all participating laboratories.

PE samples should have these general characteristics:

- Have physical, chemical, and behavioral similarities to field samples to provide an accurate test of the laboratory's procedures and method manipulations.
- Provide an evaluation of laboratory proficiency in sample analysis and quality control.
- Be homogeneous, reproducible, and stable over a required time period.
- Be appropriately represented in terms of defensible acceptance limits.
- Have scientifically valid and legally defensible certification data (for each PE sample).
- Be available on a long-term basis in sufficient and reliable supply.
- Have production methods that are not time consuming or costly.

3.2. Specific Design Considerations.

Project-specific PE samples are ideal; however, they may not be cost effective, timely to produce, or available. Therefore, generic PE samples that meet the majority of most project needs should also be considered. The remainder of this chapter discusses the following design or selection considerations:

- Matrices
- Methods
- Parameters/Analytes
- Concentrations
- Quality Assurance/Quality Control
- Homogeneity, stability, and reproducibility
- Amount

- Safety
- Cost
- Traceability, statistical design, and documentation

3.2.1. Matrices. Sample matrices may be categorized into air, water, soil, sediment, sludge, ash, oil, waste, tissue, etc. The PE samples used by USACE are mainly prepared with reagent water, clean soils, or real-world environmental matrices. Design guidelines for matrices include the following:

- Base matrix type on the sample preparation methods required for field samples and the problem at hand.
- Consider the origin, mineralogy, and pretreatment of field samples when PE samples are prepared with clean soil or real-world matrices because significant matrix differences can exist.
- Remember that USACE will also prepare PE samples with site-specific sample matrices, such as spiked, well characterized field matrices by request. However, site-specific PE samples are usually not cost effective and may not be available in a timely manner.

3.2.2. Methods. General method considerations include the following:

- Use only preparation and analytical methods that provide equivalent results. Not all sample preparation and analytical methods are equal in performance, so the acceptance limits for a PE sample based on one set of preparation and analytical methods may not be applicable to another set of preparation and analytical methods.
- Demonstrate the equivalency of method performance on each target analyte if other methods must be used. See USEPA guidance presented in USEPA/530/SW-87/003, "Test Method Equivalency Petitions," to evaluate the equivalency of test methods. Specific guidelines for analytical and preparation methods are described below.

3.2.2.1 Analytical Methods. Decide on the analytical method or instrumentation for analysis according to the nature of the field samples and sensitivity requirements. For example, analytical methods for metal PE samples depend on the metal elements, concentrations, and project requirements. They include:

- Inductively coupled plasma-mass spectrometry (ICP-MS).
- Inductively coupled plasma-atomic emission spectroscopy (ICP-AES).
- Flame atomic absorption spectroscopy (FLAA).
- Graphite furnace atomic absorption spectroscopy (GFAA).
- Cold vapor atomic absorption spectroscopy (CVAA).
- Gaseous hydride atomic absorption spectroscopy (GHAA).

PE samples prepared for a highly sensitive method, such as ICP-MS or GFAA may not be appropriate for a less sensitive method, such as ICP-AES or FLAA. Because most USACE environmental projects request USEPA SW-846 methods, the majority of USACE PE samples are designed and prepared for these methods. The same PE samples designed for SW-846 methodology can also be used for other methodologies prescribed by USEPA Superfund, Drinking Water, or Waste Water Programs. Also, a properly designed PE sample may be used for proficiency testing of multiple methods of different analytical techniques such as gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) analyses.

3.2.2.2. Preparation Methods. For each matrix-method-parameter/analyte combination, there are a number of applicable sample preparation methods including sample digestion, extraction, cleanup, and concentration procedures. Factors for choosing a method include the following:

- Different sample preparation methods, such as sonication versus Soxhlet or hot plate versus microwave, may result in different recoveries of target analytes from certain sample matrices.
- PE samples designed for one specific sample preparation method may not be appropriate for performance evaluation of another sample preparation method.
- Low target analyte concentrations caused by lower recoveries with certain preparation methods may make the PE samples not challenging or too challenging.
- The recoveries of target analytes in PE samples, especially those prepared with real-world matrices, should be empirically checked to ensure that the same analyte recoveries are achieved with different sample preparation techniques. Determine an acceptance range to encompass the diversity of techniques. In case of substantial differences in analyte recoveries among preparation techniques, set separate acceptance ranges for individual or groups of techniques.

3.2.3. Parameters/Analytes. Guidelines for PE sample parameters/analytes include:

- Make sure that a single blind PE sample of a matrix-method-parameter/analyte combination contains all or the majority of representative, method- or project-specified target analytes. The minimum number of analytes that will be present in each aqueous PE sample shall comply with the National Environmental Laboratory Accreditation Conference (NELAC) standards. A double blind PE sample, however, should not contain all target analytes because this would be so unusual as to make it recognizable as a PE sample.
- Include components that cause known analytical and preparatory interferences besides target analytes. This approach will uncover whether a laboratory performs adequate interference corrections.
- Consider as much information as possible about analytes of interest, possible interferences, and limitations of analytical methods.

Specific information about parameters/analytes in the rest of this section includes challenging analytes, false positives and false negatives, formulation strategy, and incompatible analytes.

3.2.3.1. Challenging Analytes. It is common to include both more and less challenging target analytes in a PE sample and to evaluate the performance of a laboratory on an analyte-by-analyte basis. Challenging analytes are unstable, reactive, or interfering under certain sample preparation and analysis conditions. Use these analytes to check whether a laboratory takes proper precautions and corrective actions. Examples include: breakdowns of DDT and Endrin in dirty injection ports of a GC; loss of dichlorobenzene, the most volatile of the semivolatile compounds, because of poor nitrogen blow-down technique; low recovery of phenols because of incomplete acidification of the sample, less than required extraction time, excess drying of the extract, etc.

3.2.3.2. False Positives and False Negatives. False positives or negatives are serious but common problems with environmental chemical analysis. Laboratory contamination and retention time shift are common causes of false positives and negatives of organic analysis, respectively.

Design PE samples to test the ability of a laboratory to avoid reporting false positives or negatives in these ways:

- Identify false positive problems by looking for detection of analytes that are purposely left absent.
- Identify false negative problems by adding low-level analytes and watching for non-detects. Or the PE samples may contain isomers of analytes that elute closely and possess certain common mass ions, high levels of transition metals that exhibit potentially interfering spectral lines, or excess phthalate esters or elemental sulfur that interferes with the analysis of pesticides or polychlorinated biphenyls.

3.2.3.3. Formulation Strategy. Use specific modifications of PE sample compositions to provide additional checks of the entire analytical process. For examples, see the following list:

- Semivolatile PE samples should contain acid, base, and neutral analytes over the full retention time range.
- The addition of isomeric pairs to organic PE samples checks GC resolution.
- The addition of phthalates to pesticide PE samples tests extract cleanup methods.
- The addition of oil to soil PE samples verifies whether a gel permeation chromatographic cleanup was performed as contract required.
- The use of potassium ferricyanide, instead of potassium cyanide, to prepare aqueous cyanide PE samples checks whether distillation was conducted.
- Various other analytes may be added to gauge instrument performance, such as: addition of chloromethane to volatile PE samples to check if correct purge flow was used; addition of di-n-octyl phthalate to semivolatile PE samples to determine if the temperature of GC/MS transfer line

was set too low; use of specific xylene isomers to indicate if proper standards and response factors were used to set up instrument criteria; etc.

3.2.3.4. Incompatible Analytes. Certain groups of compounds should not be combined since they will react together. Select specific reagents for each analyte on the basis of not only quantity and availability but also on chemical characteristics (i.e., stability and reactivity). Use these guidelines:

- High concentrations of semivolatile acids and bases should not be combined because they will react with each other causing subsequent loss of analytes.
- Silver and low-to-medium levels of chloride ions are incompatible and should not be mixed.
- Certain compounds may not even be compatible with some instruments and should not be used. For example, it is difficult to use GFAA to analyze PE samples with high concentrations of chloride ions because of suppression of analyte signals.
- An expiration date must be established and specified for all prepared materials including PE samples, reagents, reference standards, etc.
- PE samples that contain unstable target analytes may need to be supplied in sealed ampules or as a concentrate in nonaqueous solvents to enhance the stability of the analytes.

3.2.4. Concentrations. Concentration of PE samples should be determined using these guidelines:

- Concentrations should be near the levels expected in field samples or span the range of the analytical method.
- Concentrations may also be prepared near project or regulatory action levels to check laboratories' performance near action levels.
- Concentrations also depend on the target analytes of interest and the method of analysis. For example, the concentrations of metal PE samples depend on the metal elements, analytical methods, and project requirements. *As a general rule, to check a laboratory's performance in accuracy, the concentrations of target analytes should be at least ten times higher than sample-specific Method Detection Limits (MDL) to avoid excessive random errors near the MDL.* However, to check a laboratory's ability to avoid false negatives, the concentrations of target analytes may be set at three to five times above the MDL.
- Both high and low concentrations may be included to check if a laboratory would analyze field samples at project-required dilutions. Such multiple dilutions or concentrations may be needed to quantitate all target analytes correctly. However, avoid excess interferences between closely eluted compounds of substantial concentration difference that would require unique or non-routine treatments to pass PE samples.

3.2.5. Quality Assurance/Quality Control. Use proper design and different types of PE samples to detect and correct specific quality assurance/quality control (QA/QC) problems.

- Analytical precision can be verified by duplicate analyses of well homogenized PE samples (e.g., aqueous PE samples that are intrinsically homogeneous down to a molecular level) that contain many analytes at midrange concentrations.
- Matrix spike recovery problems can be verified by submitting a spiked field sample and a spiked extract or digest of the same field sample. Differences in recoveries between pre- and post-extraction/digestion spikes will demonstrate whether the extraction/digestion process at a laboratory is performing well.
- Precision data based on PE samples of clean matrices and real-world matrices provide information about the true laboratory precision against the precision difficulty associated with the method on complex matrices.

3.2.6. Homogeneity, Stability, and Reproducibility. In general, USACE's PE samples are individually prepared for each laboratory on an as needed basis. To assure homogeneity in representative subsamples, follow ASTM Standards D6051 and D6323. Sample homogeneity depends on the following:

- Specificity of the characteristic.
- Precision of measurement. Because aqueous PE samples are prepared by spiking target analytes into individual sample containers, reproducibility of PE samples within and between preparation batches is critical. The heterogeneity of aqueous PE samples is mostly due to differential contamination during the preparation and final packaging or incomplete dissolution or equilibration of an analyte(s) in the spiking solution.
- Defined sample size of the test portion. PE samples of solid matrices are often prepared in large quantity and subsampled for individual PE samples. Homogeneity and stability of the bulk PE materials are crucial to ensuring that equivalent samples are sent to all participating laboratories over an extended time period. Soil PE samples are heterogeneous in composition by nature but are accepted as homogeneous with respect to a specific characteristic if no difference on this characteristic among different parts can be experimentally detected.

The remainder of this section describes initial verification and ensuring equivalent PE samples for aqueous, solid, and unstable sample types.

3.2.6.1. Initial Verification. PE samples must be verified on a regular basis to show that there are no significant differences on the prepared analyte concentrations within and between batches. Prior to PE sample use follow these guidelines:

- Use generally accepted testing procedures to establish the homogeneity, stability, and reproducibility of PE samples. A suitable testing procedure should compare PE samples across production runs for comparability at " = 0.05 level using appropriate statistical testing.

- Refer to ISO Guide 35 for Certification of Reference Materials and ASTM Standards E826, D4515, D4841, and F1469 for suitable testing procedures.

3.2.6.2. Verification of Aqueous PE samples. Full volume, aqueous PE samples are freshly prepared on the day of shipment. The samples are considered stable within the method specified holding time under method-recommended storage conditions. Test guidelines for verifying aqueous PE samples include:

- Use a single holding time study for each particular type of aqueous PE sample according to ASTM Standards D4515 and D4841.
- Verify PE samples that contain unstable analytes or analytes at low concentrations with "end of holding time" sample analyses. The sample is considered stable within the holding time if the mean measured concentrations of "end of holding time" samples do not show significant differences (i.e., at " = 0.05 using a conventional *t* test) from the mean prepared concentrations based on pre-shipment verification analysis.

Ideally, PE samples should be stable for some time after the return of results by participating laboratories in case there are any queries and problems that need to be addressed.

3.2.6.3. Verification of Solid PE Samples. For soil and sediment PE samples, following these guidelines for monitoring stability:

- Verify the sample concentrations of bulk PE sample materials on a routine basis; results should fall within the 95% confidence intervals (i.e., ± 2F) of previously determined mean reported values.
- Carry out stability studies (depending on the degradation mechanisms) at elevated temperatures to accelerate the degradation rate and hence reduce the time needed to collect sufficient data.
- Obtain stability data from the manufacturers of materials or through previously published data.
- Refer to the detailed procedures for investigations of sample homogeneity, stability, and reproducibility in Section 5.3 of Chapter 5.

3.2.6.4. Unstable PE Samples. The following steps are taken for unstable PE samples to ensure equivalent PE samples:

- Provide special instructions on sample storage and treatment to participating laboratories.
- Take precautions to ensure that samples remain unchanged, at a minimum, till the end of method-specified holding time.
- Package PE samples that consist of a mixture of powders of different relative density or of different grain size carefully to avoid segregation that may result during transport.
- Seal PE samples into ampules if reactions with the atmosphere may be expected. The ampule may be filled with an inert gas.

3.2.7. Amount. Use these guidelines to determine PE sample amount:

- Provide a limited amount of PE samples so that a laboratory has only one chance to prepare and analyze them and cannot split samples and compare results.
- Use "full-volume" PE samples (unless stability is a concern) that mimic the compositions of field samples to evaluate the entire laboratory (sample handing, preparation, analysis, method selection, record keeping, and data validation and reporting).
- Use ampule PE samples of reduced volume or mass but at high concentrations if stability is a major concern. For example, use ampule PE samples for organophosphate pesticides in water and volatile organic compounds in soil.

3.2.8. Safety. Because hazardous and toxic chemicals are used to prepare PE samples, safety guidelines to consider include:

- Establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.
- Consider any hazards that the PE samples might pose and communicate them to parties that might be at risk.
- Make Material Safety Data Sheets (MSDS) available for each material and read and follow them carefully.
- Treat all unknown samples as hazardous and toxic, and handle all toxic materials in a well ventilated fume hood.
- Use a Chemical Hygiene Plan (CHP) to establish responsibilities, policies, and procedures for handling hazardous chemicals in the laboratories. (Both PE Sample Providers and participating laboratories should have a CHP.) The CHP shall comply with Occupational Safety and Health Administration (OSHA) Standard, 29 Code of Federal Regulations (CFR) 1910.1450. This EM does not purport to address all of the safety concerns, if any, associated with its use.

3.2.9. Cost. Proficiency testing is an effective but also expensive way to evaluate laboratory performance. The cost includes, but is not limited to, operation, maintenance, instrument downtime and repair costs, etc. Ways to reduce the cost of PE sample preparation include:

- Consider the potential use of a single PE sample for evaluation of multiple methods. For example, a well designed PE sample for volatile organic compounds could be used to test laboratory performance on multiple analytical methods including Methods 601, 602, 8021B, and 8260B.
- Select analyte, concentration, and packaging so that one PE sample can serve multiple purposes successfully at substantial cost reductions. For example, with proper packaging, a single PE sample may be used as a single blind or double blind PE sample.

3.2.10. Other Considerations. Other design guidelines discussed in this section include traceability, statistical design and analysis, and documentation and record keeping.

3.2.10.1. Traceability. The assigned purity or concentration value and the associated uncertainty of all chemicals, reagents, and reference standards used for preparation of PE samples should be traceable to National Institute of Standards and Technology (NIST) or other equivalent standards. The following guidelines are necessary for PE Sample Providers to follow:

- Provide evidence of the traceability for all reagents, chemicals, and standards used for PE sample preparation to known and accepted national or international certified reference materials.
- Have all measurement and testing instruments, equipment, and apparatus certified or calibrated against national or international standards to ensure the accuracy of measurements.

3.2.10.2. Statistical Design and Analysis. Statistical design and analysis play a key role in a PE Sample Program. Involve a statistician or someone with extensive statistical design and analysis experience in experiment design and data analysis of PE samples. Expected responsibilities of the statistician include:

- Participate early in the planning, design, and certification of PE samples.
- Perform critical examination of experimental data to identify and treat data gaps, outliers, or other irregularities.
- Test the suitability of the model.
- Analyze data with appropriate statistical tools.

3.2.10.3. Documentation and Record Keeping. The PE Sample Providers must:

- Establish and maintain a secure record keeping system with limited access.
- Maintain a system for logging files in and out to suit any particular circumstances that might be needed to comply with applicable requirements.
- Retain all individual measurement observations, appropriate calculations and derived data, sample preparation, instrument calibration records, and certification procedures for a minimum of five years after the discontinuance of the PE samples. (Records may need to be retained longer per standards from ISO, ASTM, ANSI, ASQ, USEPA, and USACE.)
- Prepare PE sample documentation that is complete, accurate, legible, indelible, unambiguous, and objective such that any information necessary for interpretation and reconstruction of the PE samples is available.
- Maintain an original copy of all data reports showing all corrections or changes on file.

The trend to replace hard copy documentation with electronic documentation is expanding rapidly, and the data management practices used to protect the integrity of electronic data are becoming increasingly important. Minimum records to be maintained include:

- A description of the hardware and software used.
- Written standard operating procedures that document procedures for generating, reviewing, and validating electronic data.
- Results of periodic in-house audits of electronic data generation and reporting.
- Backup files for electronic data generated in accordance with USEPA Directive 2185, "Good Automated Laboratory Practices." This document suggests appropriate frequencies for generating backup files (including system operating files and application files) and suggestions for off-site storage of software and hardware backups.
- Written certification (for electronic certification with a unique identifier) by the laboratory manager verifying the authenticity of each data report. Maintain such certifications for audits.

3.3. Customization.

Upon request, the Program Manager will provide customized PE samples that are totally site-specific in sample compositions and double blind to the laboratory.

3.3.1. Site Specificity. An ideal PE sample should be site-specific; however, total site-specific PE samples may not be cost-effective and/or available in a timely manner. The majority of USACE aqueous PE samples are tailored to meet site specificity in target analytes and concentrations; however, most solid PE samples are not site-specific due to technical and cost considerations.

3.3.2. Double Blindness. To conceal the identity of double blind PE samples, follow these guidelines:

- Pay special attention to the constituents of the PE samples during the design.
- Make the constituents, including target analytes, concentrations, interferences, matrices, etc., compatible with field samples. If the constituents of PE samples are substantially different from those of field samples, laboratories could easily identify them as PE samples.
- Use the same packaging, shipping, and documentation procedures as those of the field samples to hide the PE sample identity.
- Insert double blind PE samples with the same packaging, labels, and documentation as those of the field samples into normal field sample streams.
- Use a fictitious contract to ship double blind PE samples.